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ABSTRACT

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YS2E-1-1 Quantitative evaluation of G protein coupling with GPCRs

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To determine coupling of G proteins (Gs, Gi, Gq/11 and G12/13) is an important step toward understanding GPCRs. However, because downstream signaling of each G protein differs, it is difficult to comprehensively evaluate and compare G protein coupling of GPCRs. Here we develop a simple method to evaluate G protein coupling by utilizing chimeric Ga subunits and a recently developed TGFa shedding assay (Inoue et al. Nature Methods 2012). TGFa shedding responses occurred downstream of Gq/11 and G12/13 signaling in HEK293 cells. In Gq/11/12/13-depleted cells generated by a CRISPR-Cas9 system, GPCR-induced TGFa shedding response was completely diminished. Under this condition, we evaluated how effectively co-expression of a chimeric Gaq subunit harboring six C-terminal amino acid residues from other Ga subunits (e.g., Gaq/s, Gaq/i, etc) enhanced TGFa shedding responses upon GPCR stimulation. Using the method, we could successfully characterize G protein coupling of most GPCRs examined. We will also discuss usefulness of the newly generated G protein-deficient cells. In summary, the assay platform we developed here provides a simple, useful method to assess G protein coupling of GPCRs.

YS2E-1-2 Multimodal functions of the m2 muscarinic acetylcholine receptor

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The m2 muscarinic acetylcholine receptor (m2 mAChR) is a member of the superfamily of G-protein-coupled receptors (GPCRs). The m2 mAChR has key roles in modulating cardiac function and many central processes. This receptor has been known to preferentially couple with the Gi/Go family of G-proteins and the cellular effects caused by the receptor stimulation are mediated by both activated Gα-subunits as well as free Gβγ complexes. The past few years have witnessed new insights into m2 mAChR physiology, pharmacology, and structure. In this time, we present evidence that m2 mAChR can modulate the negative regulation of G-protein signaling by RGS protein. In order to analyze the dynamics of receptor-mediated signaling, we recorded the currents through G-protein-coupled inwardly rectifying potassium channels. We found enhanced RGS-mediated inhibition of G-protein signaling during m2 mAChR stimulation by its partial agonist, under the strong influence of membrane potential. Partial agonists for m2 mAChR may also be able to inhibit other GPCR signalling pathways by promoting RGS-mediated inhibition. Now we try to explore the structural basis of such a novel function of mAChR. New findings show the multimodal functions of m2 mAChR in both signal perception and transduction.

YS2E-1-3 The role of leukotriene B4 receptor 1 in myocardial infarction

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Leukotriene B4 receptor 1 (BLT1) is a member of G protein-coupled receptors, and is well known as a high-affinity receptor for leukotriene B4 that is a potent lipid chemoattractant for myeloid leukocytes. BLT1 is expressed on a variety of immune cells and has been implicated as a mediator of diverse inflammatory diseases. In ischemic heart diseases, inflammatory cells are rapidly recruited to the infarcted area. Recent reports have demonstrated that these cells amplify inflammatory responses, leading to worse pathological conditions. In this study, we have investigated the role of BLT1 in myocardial infarction (MI). BLT1 was up-regulated in infarcted hearts. Immunohistochemical and bone-marrow transfer experiments revealed that the upregulation of BLT1 was due to the recruitment of immune cells including monocytes and macrophages derived from bone-marrow cells. In BLT1-KO mice, the recruitment of the immune cells was attenuated, leading to less inflammation at the infracted area. In addition, we found that BLT1-KO mice exhibited improved survival and cardiac functions after MI compared to wild type mice. In summary, our results suggest that BLT1 expressed in phagocytes amplifies inflammation and worsens pathological conditions after MI.

YS2E-1-4 Agonist-independent GPCR activity regulates anterior-posterior targeting of olfactory sensory neurons

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G-protein coupled receptors (GPCRs) are known to possess two different conformations, active and inactive, and spontaneously alternate between the two in the absence of ligands. Here, we analyzed the agonist-independent GPCR activity for its possible role in receptor-instructed axonal projection. We generated transgenic mice expressing activity mutants of the beta-adrenergic receptor, a well-characterized GPCR with the highest homology to odorant receptors (ORs). We found that mutants with altered agonist-independent activity changed the transcription levels of axon targeting molecules, e.g., Neuropilin-1 and Plexin-A1, but not of glomerular segregation molecules, e.g., Kirrel2 and Kirrel3, thus, causing shifts in glomerular locations along the anterior-posterior (A-P) axis. Knockout and in vitro experiments demonstrated that G_s, but not G_{olf}, is responsible for mediating the agonist-independent GPCR activity. We conclude that the equilibrium of conformational transitions set by each OR is the major determinant of expression levels of A-P targeting molecules.

YS3E-2-1 CGRP is involved in depression-like behavior

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Changes in neurotrophic factors are involved in the pathogenesis of depression. However, the relationship between calcitonin gene-related peptide (CGRP), a pro-inflammatory neuropeptide and potent vasodilator, and depression-like behavior remains unclear. In this study, we used chronically stressed mice to investigate whether CGRP can affect behavior. After the 15-day stress exposure, mice exhibited depression-like behavior and decreased CGRP mRNA levels in the hippocampus. In addition, CGRP-deficient mice showed more depression-like behavior. Interestingly, a single CGRP administration into the brain, before the beginning of the 15-day stress exposure, normalized the behavioral dysfunctions, hippocampal neurogenesis and NGF mRNA levels in stressed mice. In the mouse E14 hippocampal cell line, CGRP treatment induced increases of NGF mRNA. The NGF receptor inhibitor K252a inhibited CGRP's antidepressant-like effects. These results suggest that CGRP expression in the mouse hippocampus is associated with depression-like behavior and changes in NGF mRNA levels. Our findings provide a novel function of CGRP, suggesting that CGRP pre-administration may protect against depression caused by a subsequent severe stressful situation.

YS3E-2-2 Functional role of cortical astrocytes in sleep/affective dysregulation under the chronic pain: Analysis by artificial control of astrocytes using optogenetics

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Chronic pain loses the motivation of patients with a decrease in their quality of life. The anterior cingulate cortex (ACC) area plays a role in an affective-motivational component of pain. On the other hand, it has been considered that astrocytes play a role in neural regulation. However, the role of astrocytes in the neural abnormality related to sleep/affective dysregulation under the chronic pain has not been yet to be elucidated fully. In this study, we investigated whether activated-astrocytes in the ACC region could be directly associated with pain-induced sleep dysregualtion. Mild noxious heat stimuli significantly changed the morphology of astrocytes in the ACC with increasing the release of glutamate at the synaptic cleft. Using an optogenetic tool with channel rhodopsin-2, we demonstrated that selective photostimulation of astrocytes in vivo triggered sleep disturbance and decreased pain threshold. These approaches provide novel evidences that astrocytic activation in the ACC may, at least in part, contribute to sleep/affective dysregulation along with chronic pain.

YS3E-2-3 Influence of early life stress on the chronic pain in maturation period

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Early life stress has recently been reported to be involved in the pathogenesis of psychiatric disorders and chronic pain in adult life. In the present study, we evaluated the effect of early life stress on baseline pain sensitivity and thermal or mechanical hypersensitivity induced by nerve injury in male and female mice.

Early life stress was induced by maternal separation and social isolation (MSSI). Mice were separated from dam and littermates for 6 h/day during postnatal days 15-21 and then were housed individually until the end of the study. At 7 weeks of age, MSSI induced depression-like behaviors in both male and female mice, but induced anxiety-like behaviors only in female mice. MSSI had no effect on thermal and mechanical sensitivity before nerve injury. At 9 weeks of age, the sciatic nerve was partially ligated to elicit neuropathic pain. MSSI enhanced nerve-injury-induced thermal and mechanical hypersensitivity in both male and female mice.

Our findings suggest that MSSI exacerbates neuropathic pain in adult male and female mice. Overall, this model may be useful for understanding the molecular mechanisms underlying the reciprocal relationship between early life stress and chronic pain.

YS3E-2-4 The mechanisms underlying abnormal pain in chronic fatigue syndrome model

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Chronic stress would bring about various symptoms such as severe fatigue, sleep disturbance, widespread pain, and cognitive dysfunctions. To reveal the mechanism underlying the symptoms we have used a multiple continuous stress (CS) model of rat. In this model, rats were housed in a cage with a low level of water for 5 days. Recently we have found that this model animal suffered from abnormal pain. The von Frey and Randall-Seritto tests were used to evaluate pain levels after CS loading. The mechanical allodynia at plantar skin and mechanical hyperalgesia at the anterior tibialis (i.e., muscle pain) were induced by CS loading. Intriguingly, no signs of inflammation and injury incidents were observed in the plantar skin and leg muscles. However, microglial accumulation and activation were observed in L4-L6 dorsal horn of CS rats. Intrathecal administration of minocycline significantly attenuated CS-induced mechanical hyperalgesia and allodynia, suggesting that microglial activation was involved in the development of abnormal pain in CS animals. Although the mechanism how CS activated microglia is obscure, similar event may occur in chronic fatigue syndrome patients who suffer from cryptogenic pain.